Original Article

Tumor Cells and Microvessels in Pediatric Brain Tumors

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ABSTRACT

Brain tumors represent the most common solid tumor in children and are the leading cause of cancer-related mortality in childhood. Despite modern advances in surgery, radiation, and chemotherapy, a significant proportion of the pediatric brain tumor patients still die of their disease or suffer from poor quality of life. Therefore, more effective and alternative therapies are still in urgent need.

Tumor cell lines derived from central nervous system tumors are critical tools for carrying out preclinical therapeutic testing and studying the biology of the disease. However, to date only few continuous pediatric brain tumor cell lines have been established.

Keywords: Pediatric brain tumor, apoptosis, angiogenesis

Pediatric Brain Tumor Epidemiology and Brief Classification

Brain tumors represent the second most common pediatric cancer and the most common solid tumor in childhood, accounting for 4.3 cases per 100,000 person-years in the United States [1]. The incidence peaks among children ages 3 to 7 years, although all ages are affected. Brain tumors, together with leukemia, are the leading cause of cancer-related mortality in children, despite being only half as frequent as leukemia in this age group. In adults and older children, most tumors are located supratentorially; in young children CNS tumors are more commonly infratentorial. Histologically, central nervous system tumors are diagnosed and graded by the World Health Organization (WHO) classification [2]. WHO classifies these tumors based on histology and malignancy into four grades, with 1 being the least malignant and 4 the most malignant. As a rough classification, brain tumors can be categorized into glial tumors and neuronal (nonglial) tumors [3].

Gliomas comprise a majority of brain tumors in childhood. Different gliomas share morphological similarities with the different lineages of glial cells, the respective tumors were named accordingly: astrocytomas resemble astrocytes, oligodendrogliomas resemble oligodendrocytes, and ependymomas share morphological similarities with ependymocytes, even

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though convincing evidence that these tumors are really derived from the respective precursors is inadequate. Neuronal tumors, a large group of brain tumors most frequently occurring in children, are dominated by embryonal tumors, and the most common of them being medulloblastoma. Medulloblastoma by definition is located in the posterior fossa and comprises the most frequent malignant brain tumor in children. The supratentorial counterpart, supratentorial primitive neuroectodermal tumor (PNET), is histologically indistinguishable from medulloblastoma. However, some evidence has shown that PNET may represent a different entity then medulloblastoma. Atypical teratoid rhabdoid tumor (AT/RT), a highly aggressive brain tumor characterized by loss of expression of the INI1 tumor suppressor gene, has morphologic features of medulloblastoma and PNET and also belongs to neuronal tumor category, but less common.

Pediatric Brain Tumors as Disorders in Normal Neuronal Development

Traditionally, neuroscientists have focused on degenerative diseases and developmental brain disorders, which can provide valuable insights into the normal development and function of the nervous system. However, advances in tumor genetics and the development of mouse brain cancer models have increasingly linked pediatric neoplasms with disordered mechanisms of normal development, supporting the model of embryonal tumorigenesis. Importantly, many of the pivotal mechanisms that are important for normal brain development are exactly those that have gone awry in brain cancer.

In 1928, Bailey and Cushing suggested that brain tumors could be classified by their microscopic resemblance to a presumed CNS cell of origin or its developmental precursor [4]. Although recent work suggests a more complex pattern, Bailey and Cushing model has remained a guiding principle for brain tumor classification [5,6]. According to this scheme, less malignant tumors resemble their normal tissue counterparts, whereas more malignant tumors resemble less differentiated precursor cells.

Normal cerebellar development is a predominantly postnatal event that involves migration, proliferation, and differentiation of multi-potent neural progenitor [7]. Disruption of normal signal transduction in this process has been associated with medulloblastoma, the most common primary malignant brain tumor of childhood. Pathways disrupted include Hedgehog (Hh) pathway, Wnt pathway and BMP pathway [8]. Among others, Hh pathway is the most studied pathway in CNS development and tumorigenesis, and is the key regulator in neural development controlling differentiation (cell-fate), tissue repair and body-segment polarity [9,10].

During cerebellum development, granule cell progenitors (GCPs) migrate from the rhombic lip over the outer layer of the cerebellar surface (external germinal layer, EGL). After a first burst of cell proliferation in the outer EGL, GCPs start a differentiation program by exiting from the cell cycle and moving into the inner EGL and further migrate inward into the internal granule layer (IGL), where differentiated granule cells resides . In this way, Hh signaling keeps the EGL GCP population in an undifferentiated state while promoting cell expansion [11,12,9]. This is an important aspect because of the relationship between cerebellar tumorigenesis and aberrant Hh signaling. Indeed, inappropriate activation of Hh pathway resulting from failure to constrain Hh signaling is responsible for the development of medulloblastoma. This appears to be caused by the Hh signaling dependent maintenance of cell cycle progression and arrest of differentiation of GCP, which results in the abnormal persistence of progenitor cells that are susceptible to malignant transformation [13].

Chemotherapy for Pediatric Brain Tumors

During the past three decades, chemotherapeutic agents have been extensively evaluated for the treatment of pediatric brain tumors in a myriad of schedules, doses, and combinations. Remarkable advances in outcome have been achieved for certain groups of children, notably those with medulloblastoma. However, improvements in survival are obtained at a high cost to quality of life. In addition, the progress for high- grade glioma is not optimal. Resistance to conventional chemotherapeutic agents appears to contribute to the poor response rate of some childhood brain tumors to chemotherapy.

Medulloblastoma

Before the 1980s, therapy for medulloblastoma and other PNETs consisted of surgery and craniospinal radiotherapy. Craniospinal radiotherapy adversely and seriously affects the cognitive function of developing children and long-term survival rates were dismal despite therapy. Two studies in the 1980s and 1990s largely changed the outcome for children with medulloblastoma: they started the stratification of patients and their therapy by risk group based on the absence or presence of drop metastases of the tumor, and the addition of chemotherapy to the treatment regimens of both risk groups [14].

For children with standard-risk medulloblastoma (no metastases), the addition of chemotherapy, most often currently consisting of cisplatin plus etoposide [15], improved event-free, 5-year survival from 60% to 80% and reduced the dose of craniospinal irradiation [16,17]. For children with high-risk medulloblastoma (metastatic disease), chemotherapy is aimed not only at directly killing tumor cells, but also at sensitizing tumor cells to radiation. Event-free, 5-year survival rates for these children have improved somewhat, from 40% to 60% [15,14]. Children with recurrent medulloblastoma present a particularly difficult therapeutic problem. Kadota et al. have been successful in attaining long-term survival in some children who underwent myeloablative chemotherapy including melphalan and cyclophosphamide followed by autologous bone marrow transplantation (ABMT) [18].

Due to the well recognition of profound and irreversible sequelae of radiation therapy to young children, efforts have made to completely avoid the use of brain irradiation, such as the "Head Start" trial. Some measure of success has been reported recently with both preserved intellectual functioning and quality of life, as well as possibly improved disease-free survival, suggesting that a subset of children can be successfully treated with chemotherapy alone [19].

Gliomas

Typically, surgery remains the mainstay of treatment of pediatric low-grade gliomas (mainly pilocytic astrocytoma and diffuse astrocytoma). Sometimes where tumors are not surgically accessible and to defer radiotherapy and its adverse effects, especially in infants and young children, chemotherapy is now the front-line adjuvant therapy used, and carboplatin and vincristine are often the drugs of choice [20,21].

High-grade gliomas are a heterogeneous group of tumors, the most of malignant are glioblastoma (grade IV) and anaplastic astrocytoma (grade III). In spite of modern aggressive multimodality therapy including surgery, radiationtherapy, and chemotherapy, survival remains dismal [22]. Maximum safe surgical resection and radiationtherapy are the backbone of therapy for pediatric high- grade glioma. In contrast, the role of chemotherapy for the treatment of these neoplasms remains undefined. Despite decades of intensive investigation, no single chemotherapeutic regimen stands out as particularly [23]. In the world of adult high-grade gliomas, a pivotal discovery was made in a study evaluating the drug temozolomide. Unfortunately, the trials utilizing temozolomide in children have not been as successful [24,25]. A newer strategy used in recurrent adult high-grade gliomas has been the combination of bevacizumab and irinotecan [26]. Bevacizumab is a monoclonal antibody against vascular endothelial growth factor (VEGF) and was approved by FDA for use in treating adult glioblastoma multiforme (GBM).Ongoing studies are underway evaluating this combination carefully in both adult and pediatric high-grade glioma, although some disencouraging result just recently came out [27]. The marked differences in response to temozolomide between adults and children with high-grade glioma highlight the distinct underlying biology between these groups.

Tumor Angiogenesis

Angiogenesis is a vital, highly regulated physiological process involving the growth of new blood vessels from pre-existing vessels, while vasculogenesis is the term for de novo formation of blood vessels from bone marrow-derived endothelial precursor cells. During vasculogenesis, angioblasts proliferate and assemble into a primitive network of vessels known as the primary capillary plexus. The endothelial cell lattice created by vasculogenesis then serves as a scaffold

for angiogenesis. After the primary capillary plexus is formed, it is remodeled by the sprouting and branching of new vessels from pre-existing ones- the process of angiogenesis [28]. Most normal angiogenesis occurs in the embryo, although angiogenesis also occurs in the adult during the ovarian cycle and in physiological repair processes such as wound healing [29].

Angiogenesis involves multiple, coordinated steps that can be briefly summarized as follows: 1. A cell activated by stimuli such as lack of oxygen or tumor cells programmed to express certain growth factors releases pro-angiogenic molecules that attract inflammatory and endothelial cells and promote their proliferation. 2. During their migration, inflammatory cells also secrete molecules that intensify the angiogenic stimuli. 3. The endothelial cells that form the blood vessels respond to the angiogenic call by differentiating and by secreting matrix metalloproteases (MMP), which digest the blood- vessel walls to enable them to escape and migrate toward the site of the angiogenic stimuli. 4. Several protein fragments produced by the digestion of the blood-vessel walls intensify the proliferative and migratory activity of endothelial cells, which then form a capillary tube by altering the arrangement of their adherence-membrane proteins. 5. Finally, through the process of anastomosis, the capillaries emanating from the arterioles and the venules will join, thus resulting in a continuous blood flow.

Vascular Endothelial Growth Factor (VEGF)

Angiogenesis requires the coordinated action of a variety of positively- and negatively- acting factors: among these are soluble polypeptides, cell-cell and cell-matrix interactions, and hemodynamic effects. So far, vascular endothelial growth factor (VEGF) is perhaps the most studied and best characterized angiogenic factor in developmental angiogenesis. VEGF is a highly conserved disulfide-bonded dimeric glycoprotein. It is expressed at low levels on a wide variety of human and animal tissues, but high levels are produced where angiogenesis is required such as fetal tissue, the placenta, and the corpus luteum and in a vast majority of human tumors [30,31]. In vitro, VEGF exerts several effects on vascular endothelial cells: VEGF stimulates vascular endothelial cell proliferation, inhibits apoptosis. VEGF increases endothelial cell permeability which allows the extravasation of plasma proteins and formation of extracellular matrix favorable to endothelial and stromal cell migration. Also, VEGF stimulates endothelial cell the production of plasminogen activators (u-PA and t-PA), plasminogen activator inhibitor-1 (PAI-1) [32]. Therefore, VEGF induces a balanced system of proteolysis that can remodel extracellular matrix components necessary for angiogenesis. In vivo, many experiments also implicate VEGF in angiogenesis. In the cornea and healing bone grafts, VEGF induces growth of capillary sprouts from pre- existing blood vessels [33]. Mice deficient in the gene for VEGF and the VEGF receptor Flk-1 could be embryonic lethal and are virtually devoid of vascular structures, and are thus defective in the very early events in blood vessel formation that characterize vasculogenesis [34,35,36].

Fibroblast Growth Factor (FGF)

Basic and acidic fibroblast growth factors are ubiquitously expressed polypeptides that are members of a large family of structurally related growth regulators and have been thought to play a role in normal angiogenesis. In fact, acidic FGF was the first growth factor to be associated with angiogenesis [37]. Similar to VEGF, both bFGF and aFGF induce processes in endothelial cells in vitro that are critical to angiogenesis. FGF also stimulates proliferation of most, if not all, cells derived from embryonic mesoderm and neuroectoderm, including pericytes, fibroblasts, myoblasts, chondrocytes, and osteoblasts [38]. However, it appears that aFGF and bFGF do not play a major role in angiogenesis in vivo because vascular development is normal or only show mild in mice deficient in both aFGF and bFGF [39,40]. Some recent findings suggest that FGF-induced angiogenesis requires activation of the VEGF system [41].

PDGF and Ang

Other signaling molecules that have a well established role in the development and differentiation of the vessel wall are platelet derived growth factors (PDGF) and the angiopoietins (Ang), the ligands of the Tie2 receptor

[42]. PDGFs are homodimers (PDGF-AA or –BB) or heterodimers (PDGF-AB) composed of PDGF chains A and B. PDGF receptors are also dimeric in nature; they are made up of complexes between α and β subtypes ($\alpha\alpha$, $\alpha\beta$, $\beta\beta$). In vitro, PDGF-BB stimulates DNA synthesis and the formation of angiogenic chords and sprouts on capillary endothelial cells [43,44]. PDGF has also been shown to be important for angiogenesis in vivo. Mice deficient in PDGF-B or PDGF receptor-β develop die perinatally [45]. Electron microscopic analysis of PDGF-B-deficient mouse brain capillaries shows the absence of pericytes and dilated vessel lumen. Specific inhibition of PDGFRβ signaling eliminates mature pericytes around tumor vessles, leading to vascular hyperdilation and endothelail cell apoptosis [46]. Therefore, PDGF may play a critical role in recruitment of pericytes to preformed capillaries or in inducing the proliferation of pericytes previously recruited by a PDGF-independent mechanism, and it thus helps to maintain capillary wall stability [47].

The angiopoietins are secreted ligands for Tie2. Angiopoietin-1 (Ang1), the most extensively characterized member of this family, is required for further remodeling and maturation of the initially immature vasculature. Unlike mouse embryos lacking VEGF-A, embryos lacking Ang-1 or its receptor Tie2 develop a rather normal primary vasculature, but this vasculature fails to undergo effective remodeling [42]. The most severe defects are in the heart, where endocardial and trabecular development is notably impaired.

Integrins

In addition to factors that are secreted from cells and act at a distance from their sites of synthesis, several membrane-bound proteins, such as integrins also play prominent roles in angiogenesis. Integrins are heterodimeric complexes composed of α and β subunits that are receptors for extracellular matrix proteins and membrane-bound polypeptides on other cells. Over 18α and 8β subunits can combine to form a diverse array of over 24 different integrins [48]. Extracellular matrix substrates for integrins such as fibronectin, vitronectin, collagen, laminin, and elastin [49]. A number of endothelial cell integrins regulate angiogenesis in diverse manners, including integrins $\alpha1\beta1$, $\alpha2\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, $\alpha9\beta1$ and $\alpha6\beta4$ and αv integrins. In particular, antagonists of integrin $\alpha v\beta3$, $\alpha v\beta5$ and $\alpha5\beta1$ are currently under investigation to suppress tumor angiogenesis. Integrin $\alpha v\beta3$ is abundantly expressed on angiogenic blood vessels, and it mediates endothelial cell attachment, spreading, and migration in vitro. Some integrins bind short peptide sequences RGD (Arg-Gly-Asp) sequence found in fibronectin and vitronectin [50]. On ligation to the ECM, integrins cluster in the plane of the membrane and recruit various signaling and adaptor proteins to form structures known as focal adhesions. Although integrins lack kinase activity, by clustering they recruit and activate kinases, such as focal adhesion kinases (FAKs) and Src family kinases (SFKs), in addition to scaffolding molecules, such as p130 CRK-associated substrate (p130CAs). Integrins also couple the ECM to the actin cytoskeleton by recruiting proteins, including talin, paxillin, actinin, tensin and vinculin. The role of $\alpha v\beta3$ in blood vessel growth has also been examined in embryonic vascular development. αv integrin knockout mice exhibit vessel abnormalities and hemorrhaging in brain and intestinal vasculature [51], and mice deficient in $\beta3$ integrins exhibit extensive bleeding due to abnormal platelets but demonstrate grossly normal vasculatures [52].

Angiogenesis in Neuronal Development and Neuropathology

Glia and neurons in the CNS lie a network of blood vessels. In the absence of proper CNS vascularization, the neuroepithelium undergoes apoptosis followed by progressive tissue destruction, especially at the subventricular zone leading to embryonic lethality [53]. The vasculature of the CNS develops exclusively via angiogenesis but not by de novo vasculogenesis as seen in other tissues. In early embryogenesis, vessels from the pia mater, which closely envelops the entire surface of the brain, invade the brain and converge centripetally towards the ventricles. This is followed by extensive branching and arborization as capillary sprouts migrate from the pia surface to periventricular areas, where angiogenic growth factors such as VEGF are highly expressed and secreted by cell in

the subventricular zone [54]. This process continues through the remainder of embryonic development in all CNS tissues. Once the vascular plexus has migrated into the brain, the blood vessels of the CNS undergo vascular remodeling and pruning coupled with endothelial cell recruitment of vascular smooth muscle cells (VSMCs) to form the mature functional CNS vasculature. CNS vasculature also plays a vital role in many common human disorders such as stroke, motor neuron degeneration, cancer and autoimmune disease. Among the pathological conditions of the CNS vascular network, stroke is a major disease in which the supply of blood is decreased due to obstruction of large or mid-size vessels. These lesions result in severe ischemia of neurons and astrocytes around and downstream of the lesions, eventually inducing necrotic cell death. Increase in vascular density is observed in the stroke area after the stroke, and this increase in blood flow can rescue the ischemia and help the recovery from the stroke [54].

Tumor Angiogenesis

Tumor angiogenesis is the tumor-induced growth and remodeling of blood vessels. In order for tumors to develop a more malignant phenotype, they require oxygen and nutrients, which are supplied by blood vessels [55]. Without an efficient blood supply, solid tumors cannot grow beyond 1-2 mm in diameter [56], thus the initiation of tumor angiogenesis, termed the "angiogenic switch", is critical for solid tumor growth and progression [57]. The induction of angiogenic switch depends on the balancing between pro-angiogenic factors and anti-angiogenic factors. Pro-angiogenic gene expression is increased upon physiological stimuli, such as hypoxia induced by increased tissue mass [58], and also by oncogene activation or tumor-suppressor mutation.

The tumor vasculature is very different from "normal" blood vessels where the vessels are tightly regulated and quiescent after maturation. Tumor vessels, however, fail to become quiescent and undergo constant growth. Thus, tumor vessels develop some characteristics that are very distinct from normal vasculature. There are architectural, phenotypic, and functional differences, which allow for the straightforward distinction between the normal and tumor endothelium. Tumor blood vessels lack organization into arteries, capillaries, and veins. They are irregularly shaped, dilated, tortuous and can have dead ends. They are often leaky and haemorrhagic, mainly due to the increase in tissue pressure, stasis of blood causes by tortuous vessels, and overproduction of VEGF. Furthermore, the tumor endothelium has poor association with the basement membrane and mural cells [59].

In the brain, normal brain vasculature is highly specialized. Endothelial cells, pericytes and astrocytes all together form blood-brain barrier (BBB) which is a separation selectively restricts the exchange of molecules between blood and cerebrospinal fluid (CSF). When the tumor begins to grow beyond 1–2 mm in diameter within the brain parenchyma, normal brain vasculature is disrupted, featured by loss of BBB integrity both structurally and functionally, marked angiogenesis with endothelial proliferation, severe hypoxia and tumor necrosis [60,61,62,63]. So far, most of the available data in the literature on brain tumor angiogenesis are from studies of malignant gliomas. For example, glioblastoma vessels are tortuous, characterized by abnormalities in their endothelial wall, pericyte coverage and basement membrane; the vessels have pores in the walls which have larger diameters and thicker basement membranes [60,64,65]. This abnormal structure leads to abnormal in vascular function. An uneven increase of vascular permeability is observed, characterized with loss of permselectivity [66]. The heterogeneous leakiness of tumor vessels causes an abnormal blood flow. Focal leaks can contribute to non-uniform blood flow and heterogeneous delivery of oxygen and blood-borne drugs [67].

Tumor angiogenesis is a multi-step process that is orchestrated by a range of angiogenic factors and inhibitors. VEGF is a major permeability and pro-angiogenic factor that is highly expressed in brain tumors and is partly responsible for the loss of the BBB during tumor growth. Hypoxia and acidosis have been shown to independently regulate VEGF transcription in brain tumors [68]. Neovascularization of brain tumors, particularly in gliomas, is thought to be driven mainly by VEGF signaling [69]. Cell-matrix receptors such as

the $\alpha v\beta 5$, $\alpha v\beta 3$ and $\alpha 5\beta 1$ integrins, which mediate endothelial-cell spreading and migration in response to growth factor signaling, also play a critical role in the maintenance of new vessels during tumor angiogenesis [70].

Anti-angiogenesis Therapy for Brain Tumors

The hypothesis that inhibition of angiogenesis (antiangiogenesis) would be an effective strategy to treat human cancer was first brought up in 1971 by Dr. Folkman, the pioneer of angiogenesis and antiangiogenic therapy [56]. With this idea around for thirty years, the demonstrated dependence of tumor growth and metastasis has provided a powerful rationale for antiangiogenic approaches to cancer therapy [71]. Targeting blood vessels in brain tumors has been a particularly attractive strategy, given the characteristic high degree of endothelial proliferation, vascular permeability, and pro- angiogenic growth-factor expression in the brain [72,73].

Several models have been proposed to explain the mechanisms of antiangiogenic drugs, both as single agent and as a combination agent with chemosensitizing activity. First and foremost, antiangiogenic agents lead to disruption tumor-associated vessels. The inhibition or cytoxic effect on vessels starves the tumor, resulting in its shrinkage or stabilization in size. Second, antiangiogenic drugs prevent rapid tumor cell repopulation after cytotoxic chemotherapy. Treatment of most conventional cytotoxic drugs initially induces tumor shrinkage, but this is almost always followed by tumor cell repopulation. Antiangiogenic agents could be administered during the break period between courses of chemotherapy when most tumor cell repopulation occurs. Third, antiangiogenic drugs ''normalize'' the tumor vasculature which can both shrink tumor and enhance the efficacy of chemotherapeutic drugs [74].

Many antiangiogentic compounds are currently being explored in both clinical and preclinical phases. In 2004, the first antiangiogenic drug, bevacizumab, a humanized monoclonal antibody directed against VEGF-A, was approved by the United States Food and Drug Administration (FDA) for the first-line therapy of metastatic colorectal cancer in combination with 5-fluoro-uracil and leucovorin. Moreover, bevacizumab was approved for the treatment of GBM in May 2009.

Apoptosis and its Role in Cancer Therapy

Apoptosis is the process of programmed cell death (PCD), a highly phylogenetically conserved mechanism by which eukaryotic cells die following a series of molecular and cellular events. In 1972 the term apoptosis was first introduced on morphological grounds [75]. However, after that, accumulating evidence suggests that PCD is not confined to apoptosis---PCD is the phenomena observed and apoptosis is one of the mechanisms. Later, three morphologically distinct types of cell death in the developing embryo was defined, which we know as apoptosis, autophagy and necrosis [76].

Cells die in response to a variety of stimuli and during apoptosis they do so in a controlled, regulated fashion. This makes apoptosis distinct from necrosis in which uncontrolled cell death leads to lysis of cells, inflammatory responses and release of harmful chemicals into the surrounding tissue. Apoptosis, by contrast, is a process in which cells play an active role in their own death, which is why it is referred as cell suicide. This cell suicide usually confers advantage in a multicellular organism—the number of cells in a multicellular organism is controlled not only by the rate of cell division, but also the rate of cell death. Apoptosis is important in controlling cell number and proliferation as part of normal development.

Apoptotic cells are featured with stereotypical morphological changes: the cell shrinks, shows deformation and looses contact to its neighbouring cells. Its chromatin condenses, the plasma membrane is blebbing or budding, and finally the cell is fragmented into compact membrane-enclosed structures, called "apoptotic bodies". The apoptotic bodies are engulfed by macrophages and thus are removed from the tissue without causing an inflammatory response. These morphological changes are a consequence of molecular and biochemical events occurring in the apoptotic cells, such as the activation of proteolytic enzymes which mediate the cleavage of DNA.

Molecular Mechanism of Apoptosis

Apoptosis can be initiated through many different stimuli, but downstream converge on a restricted number of common effector pathways [77]. Basically, apoptosis can be trigger through two main pathways, death receptor pathway (external pathway) and mitochondrial pathway (internal pathway). Both are responsible for the activation of caspase cascade and the two meet at level of caspase 3 (executioner caspase). Caspase 3 activates death substrates (CAD or DFF40) after cleavage of ICAD (inhibitor of caspase activated DNase), thereby inducing DNA fragmentation and apoptotic cell death [78].

Death Receptor (Extrinsic) Pathway

The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor- mediated interactions. This involves death receptors which belong to tumor necrosis factor (TNF) receptor gene superfamily [79]. To date, the best- characterized ligands and corresponding death receptors include FasL/FasR (Apo-1 or CD95), TNF-α/TNFR1, Apo3L/DR3, Apo2L/DR4 and Apo2L/DR5 [80].

Death receptors contain an intracellular death domain (DD), which upon ligand binding associates with an adaptor protein called Fas-associated death domain (FADD) directly or indirectly via TNFR-associated death domain (TRADD) [81,82]. FADD interacts with pro-caspase-8 to form a complex at the receptor called the death inducing signaling complex (DISC). Once assembled, DISC induces the activation of caspase-8, which eventually activates the effector, caspase 3. The BH3 only protein Bid is cleaved by pro-caspase 8 and translocates to the mitochondria to activate the intrinsic pathway [83].

Mitochondrial (Intrinsic) Pathway

In the death receptor pathway, apoptosis is triggered by a relatively small number of structurally-related ligands, such as FasL. Mitochondrial apoptosis, however, can be triggered by a variety of structurally-unrelated agents. This implies that mitochondrial apoptosis can be induced by more than one mechanism, including DNA damage, ischaemia and oxidative stress, etc.

The mitochondrial pathway of apoptosis begins with the permeabilisation of the mitochondrial outer membrane. The mechanisms through which this occurs remain controversial, however, it is thought that permeabilisation can be either permeability transition (PT) pore dependent or independent [84]. Permeabilized mitochondrial outer membrane causes dissipation of the proton gradient and the membrane become non-polarized, which results in swelling of the intermembranal space and release of apoptogenic proteins. Released proteins include cytochrome c, apoptosis inducing factor (AIF), Smac, Omi and endonuclease. Cytochrome c together with apoptosis protease activating factor (APAF-1) and pro- caspase 9 form an "apoptosome" [85]. This complex promotes the activation of caspase 9, which in turn activates effector caspases that collectively orchestrate the execution of apoptosis.

Apart from two abovementioned caspase-3-mediated apoptotic pathways, there is also other, such as caspase-12-mediated pathway activated by the calcium ions stored in endoplasmic reticulum. Although apoptosis is usually a caspase-dependent process, it may also be unrelated to caspases but mediated by other enzymes.

Regulators of Apoptosis Signaling

Although all cells are intrinsically programmed to have the ability to self-destruct, yet the process of apoptosis is tightly controlled. Actually, cell death is continuously repressed by survival signals such as signals from growth factors and hormones, etc. Those survival signals enhance the expression and/or activity of antiapoptotic regulatory molecules thereby keeping in check the activation of proapoptotic factors [86]. Also, proapoptotic factors counteract inhibitory molecules when apoptotic demise of a cell is timely and imperative. The most important of these regulators identified are Bcl-2 family and p53.

Bcl-2 Family

Mitochondrial membrane permeability is primarily regulated by proteins from Bcl-2 family, which reside on the mitochondrial outer membrane. Bcl-2 family members can be divided into antiapoptotic (Bcl-2, Bcl-

XL, Bcl-w, Mcl-1, A1, Boo, NR-13) and proapoptotic proteins (Bax, Bak, Bok, Bcl-Xs, Bid, Bad, Bik, Blk, Hrk, Bim, NIP3, Nix, NOXA, PUMA, Bmf). Most anti-apoptotic members contain the Bcl-2 homology (BH) domains 1, 2 and 4, whereas the BH3 domain seems to be crucial for apoptosis induction. The pro-apoptotic members can be subdivided into the Bax subfamily (Bax, Bak, Bok) and the BH3-only proteins (Bid, Bad and Bim) [87].

Although the impact of Bcl-2 family members on apoptosis is well known, the biochemical mechanism of their function is not entirely clear. One model claims that Bcl-2 members might directly control caspase activation [88], whereas another model proposes that they mainly act by guarding mitochondrial integrity [89]. According to the second model, in viable cells the pro-apoptotic Bcl-2 family members Bax, Bak, and BH3-only proteins are antagonized by antiapoptotic members such as Bcl-2. Upon apoptotic stimuli, BH3-only members are activated. Activated BH3-only proteins prevent anti-apoptotic Bcl-2 members from inhibiting pro- apoptotic members. In addition, they might directly induce a conformational change of Bax and Bak which subsequently oligomerize and insert into the mitochondrial membrane where they form pores either by themselves or by associating with the permeability transition pore complex. In consequence, proapoptotic factors are released from the inner mitochondrial membrane into the cytosol, such as cytochrome c which contributes to the formation of the apoptosome and the subsequent activation of the caspase cascade.

p53

p53 functions as a transcription factor regulating downstream genes important in DNA repair, cell cycle arrest, and apoptosis. The critical role of p53 is evident by the large number of tumors that bear a mutation in this gene. Loss of p53 in many cancers leads to genomic instability, impaired cell cycle regulation, and inhibition of apoptosis. After DNA damage, p53 holds the cell at a checkpoint until the damage is repaired. If the damage is irreversible, apoptosis is triggered. Induction of p53 by pro-apoptotic stimuli results in apoptosis mediated by the mitochondrial pathway. Normally, p53 is maintained at low levels by murine double minute-2 (MDM2) or the human homologue (HDM2), which inhibits the transcriptional activity of p53 and promotes degradation of p53 via the proteosome [90]. Activation of p53 involves stabilization of the protein by post-translational modifications, which disrupts the interaction between p53 and MDM2. p53 drives the expression of APAF-1 and certain pro-apoptotic Bcl-2 family members as well as eliciting transcriptional independent death pathways [91] [92]. p73 and p63 are recently discovered p53 homologues, which also play a role in apoptosis through the transactivation of certain p53 target genes [93].

IAPs

Inhibitors of apoptosis proteins (IAPs) are a family of functionally- and structurally-related antiapoptotic proteins that serves as inhibitors of apoptosis. So far, eight human IAP homologues have been identified, Cp-IAP and Op-IAP, NAIP, c-IAP1, c-IAP2, XIAP and survivin. The best characterized IAP is XIAP, which binds to caspase 9, caspase 3 and caspase 7, thereby inhibiting their activation and preventing apoptosis.

Apoptosis in Cancer Therapy

The primary causes of cancer are dysregulated proliferation that leads to cellular expansion and failing to appropriately induce apoptosis or cell death. Chemotherapy drugs that designed to perturb the proliferation usually result in damage to normal cells, and thus limit their clinical efficacy. At the practical level of battling cancer, most common cancer therapies such as chemotherapy and radiation is designed to induce apoptosis in tumor cells.

Indirect manipulation of apoptosis

Traditional chemotherapy agents such as DNA damaging drugs and mitotic inhibitors are all cytotoxic and directly affect either DNA or cell cycle. Being around for around 60 years, they make a great contribution to the improvement of overall survival rate of cancer patients [94]. These drugs indirectly manipulate apoptosis by driving pro-apoptotic signaling through the induction of cell damage.

Direct Manipulation of Apoptosis

Many new agents offer more direct targeted manipulation of apoptosis. These can be approached by through inhibition or down regulation of antiapoptosis activity, or through stimulation or upregulation of apoptosis.

Inhibition of Anti-apoptotic Signaling as Treatment Strategy

Increased levels of anti-apoptotic molecules might be essential to keep cancer cells above the apoptotic threshold. Indeed, overexpression of Bcl-2 family proteins, IAPs was observed in various types of cancers. Preclinical studies have demonstrated that downregulation of Bcl-2, XIAP and survive can trigger cell death in various cancer cell lines and xenografts, and can increase sensitivity to cytotoxic chemotherapy or radiotherapy [95]. To date, three main therapeutic strategies have been developed to block antiapoptotic signaling: antisense oligonucleotides, small- molecule inhibitors or mimetics and proteasome inhibitors. The targets include Bcl-2 family protein (Bcl-2, Bcl-w, Bcl-x, Mcl-1), survivin, XIAP, IAP1 and IAP2. So far, small-molecule inhibitors of specific anti-apoptotic regulators yielded substantial potential clinically. Antisense approaches have had suboptimum results to date, which might be due to drug delivery rather than target relevance.

Direct pro-apoptotic stimulation as a treatment strategy

Recombinant TNF-α is licensed in Europe for the treatment of peripheral sarcomas and melanomas. However, the efficacy was not optimal because of severe toxic effects. After the failure of recombinant TNF-α, therapeutic anticancer death-receptor agonism is most actively being explored by approaches focused around TRAIL [96]. TRAIL form soluble homotrimer and binds to cell-surface death receptor TRAIL- R1 and TRAIL-R2. Two main therapeutic approaches have been developed as direct proapoptotic strategies, involving the extrinsic pathway: TRAIL-receptor agonist antibodies; and recombinant ligands. Over 10 agents are being investigated in clinical trial and results are promising.

Conclusion

Pediatric brain tumor is the second frequent cancer in childhood and leading cause of cancer motility in this age group. Remarkable advances in outcome have been achieved for certain groups of children, however poor progress in high-grade glioma and resistance to conventional chemotherapeutic agents remain big hurdles in the successful treatment of the disease. Targeting blood vessels has been an attractive strategy given the special characteristics of brain tumors.

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